Inhibiting RRM2 to enhance the anticancer activity of chemotherapy

Yaqiong Zhan[a], Lushun Jiang[a], Xuehang Jin[a], Shuaibing Ying[a], Zhe Wu[a], Li Wang[b], Wei Yu[a], Jiepeng Tong[a], Li Zhang[a], Yan Lou[a], Yunqing Qiu[a,*]

[a] *State Key Laboratory for Diagnosis and Treatment of Infectious Disease, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang Provincial Key Laboratory for Drug Clinical Research and Evaluation, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 QingChun Road, Hangzhou, Zhejiang 310000, People's Republic of China*

[b] *The Department of Rehabilitation Medicine, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 QingChun Road, Hangzhou, Zhejiang, 310000, People's Republic of China*

ARTICLEINFO

Keywords:
Ribonucleotide reductase subunit M2 Chemotherapy
Chemoresistance
Small interfering RNA RRM2
inhibitors
Kinase inhibitors

ABSTRACT

RRM2, the small subunit of ribonucleotide reductase, is identified as a tumor promotor and therapeutic target. It is common to see the overexpression of RRM2 in chemo-resistant cancer cells and patients. RRM2 mediates the resistance of many chemotherapeutic drugs and could become the predictor for chemosensitivity and prognosis. Therefore, inhibition of RRM2 may be an effective means to enhance the anticancer activity of chemotherapy. This review tries to discuss the mechanisms of RRM2 overexpression and the role of RRM2 in resistance to chemotherapy. Additionally, we compile the studies on small interfering RNA targets RRM2, RRM2 inhibitors, kinase inhibitors, and other ways that could overcome the resistance of chemotherapy or exert synergistic anticancer activity with chemotherapy through the expression inhibition or the enzyme inactivation of RRM2.

1. Introduction

Ribonucleotide reductase (RNR) is the only enzyme that catalyzes de novo formation of deoxyribonucleotides and is thus a key enzyme in DNA synthesis. There are two kinds of subunits of RNR and they constitute the form of tetramer (α2β2 complex) to exert catalytical activity [1]. Ribonucleotide reductase subunit M1 (RRM1) is the large one which is encoded by the gene *RRM1*, whereas the small subunit has two isoforms, RRM2 and RRM2B, encoded by distinct genes, *RRM2* and *P53R2* in human. RRM1–RRM2 holoenzyme contributes deoxyribonucleoside triphosphates (dNTPs) to S-phase nuclear DNA replication and repair in proliferating cells, whereas RRM1–RRM2B not only provides dNTPs for nuclear DNA repair in quiescent cells but also mitochondrial DNA replication and repair [2,3].

High expression of RRM2 is common in cancers, and RRM2 is identified as a tumor promotor and cancer therapeutic target [4]. RNR inhibitors inactivate the enzyme targeting RRM1, RRM2 or their assembly, and have already been used in the cancer treatment either as a single agent or combined with other therapies [5]. In this review, we focus on RRM2 inhibitors that inactivate the RNR. There is the clinical experience for three RRM2 inhibitors, hydroxyurea, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine, 3-AP), and GTI2040. Hydroxyurea has a 30-year history as a cancer therapeutic agent and inactivates the RNR by reducing the free radical and the iron center of RRM2 [6]. However, the resistance to hydroxyurea has readily developed, limiting its usefulness. 3-AP, another RRM2 inhibitor relies on iron chelation, shows 100–1000 fold higher inhibitory potency on cancers than hydroxyurea and full activity against hydroxyurea- and gemcitabine-resistant cells [7].The RRM2 antisense oligonucleotide, GTI2040, shows strong antitumor activity in animal models and has been evaluated in phase I trials [8]. It was also demonstrated that the overexpression of RRM2 was involved in the acquisition and development of resistance to multiple chemotherapeutic drugs such as gemcitabine, doxorubicin (DOX), imatinib, and so on [9–11]. And clinical trials try to find the optimal and specific combination chemotherapy regimen using RRM2 inhibitors for cancers. A phase I trial was initiated to determine the safety profile and maximum tolerated doses of 3-AP and gemcitabine when used in combination in patients with advanced cancer [12]. And another phase I study of prolonged infusion of 3-AP in combination with fixed-dose rate gemcitabine showed evidence of preliminary activity in this heavily pretreated patient population, including prior gemcitabine failure [13]. 3-AP may restore platinum-sensitivity in platinum-resistant ovarian cancer and a partial response was seen in one of six women with platinum-resistant ovarian cancer underwent 3-AP and cisplatin infusions in a small sample clinical trial [14]. 3-AP can also increase sensitivity of chemotherapy and radiation therapy in cervical cancer cells. Clinical trials demonstrated that 3-AP treatment combined with cisplatin and pelvic radiation was well tolerated and produced high rates of clinical and metabolic responses in women with

Abbreviations: RRM2, Ribonucleotide reductase subunit M2; RRM2b, Ribonucleotide reductase subunit M2b; RRM1, Ribonucleotide reductase subunit M1; 3-AP, Triapine, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; RNR, Ribonucleotide reductase; dNTPs, deoxyribonucleoside triphosphates; CDK, cyclin-dependent kinases; 3′UTR3′, untranslated region; EGFR, epidermal growth factor receptor; AKT, protein kinase B; ERK, extracellular regulated protein kinases; siRNA, small interfering RNA; ITGB1, integrin beta1; HIF-1, hypoxia inducible factor-1; STAT, signal transducer and activator of transcription; AP-1, activator protein 1; CREB, cAMP-response element binding protein; NF-κB, nuclear factor kappa-B; Chk1, Checkpoint kinase 1; ATR, Ataxia telangiectasia-mutated and Rad3-related; LncRNA, Long non-coding RNA; 5-FU, fluorouracil; NF-Y, nuclear factor Y; HDAC, histone deacetylases; Smac, second mitochondria-derived activator of caspases; NSCLC, non–small cell lung cancer; DOX, doxorubicin; Didox, 3,4-dihydroxy-benzohydroxamic acid.

* Corresponding author.
E-mail address: qiuyq@zju.edu.cn (Y. Qiu).

https://doi.org/10.1016/j.biopha.2020.110996
Received 16 August 2020; Received in revised form 28 October 2020; Accepted 1 November 2020
Available online 20 November 2020
0753-3322/© 2020 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

cervical and vaginal cancers [15–17]. As for GTI2040, it is disappointing to see no significant benefit in the phase II trial when combined with capecitabine, docetaxel, and prednisone for the treatment of patients with renal cell carcinoma, non-small cell lung cancer (NSCLC) and castration-resistant prostate cancer [18–20]. However, the hope for GTI2040 as an adjuvant chemotherapeutic drug still remains high and clinical trials of GTI2040 combined with gemcitabine, cytarabine, and other chemotherapeutic drugs are still going on [21,22].

RRM2 overexpression is also common and vital in various cancers that resist to chemotherapy. The upregulation of RRM2 influences complex signaling pathways to mediate survival, proliferation, apoptosis, and chemoresistance of cancer cells. Therefore, RRM2 could become a potential target to overcome chemoresistance and inhibiting the expression or activity of RRM2 may be a useful adjuvant chemotherapy by enhancing cell apoptosis, suppressing cell proliferation, and interfering DNA replication and repair. In addition to the RRM2 inhibitors like 3-AP which is in clinical trials, many new approaches such as small interference RNA and kinase inhibitors are also under basic and clinical research to find better solutions of RRM2 inhibition. This review tries to explain the mechanicals of RRM2 overexpression in chemo- resistant cancers and summarize the means to enhance the anticancer activity of chemotherapeutic drugs targeting RRM2.

2. Overexpression of RRM2 is common and vital in chemoresistance

RRM1 and RRM2 work together on enzyme activity and other physio pathological activities. Why do we pay more attention to RRM2 than RRM1? As we know, gemcitabine is one of the first nucleoside analogs targeting RRM1 to be clinically recommended for therapy in pancreatic cancer, NSCLC, breast cancer, bladder cancer, and ovarian cancer, but all of these cancers have eventually developed resistance to gemcitabine [9,23,24]. Resistance to gemcitabine occurs most often through modification of gemcitabine metabolism with altered activities of enzymes involved in the metabolism of the drug, of target enzymes, and of enzymes involved in programmed cell death [25]. In fact, the overexpression of both RRM1 and RRM2 was found could establish a pancreatic cancer cell line with acquired gemcitabine resistance [26]. As gemcitabine targets RRM1, but RRM2 also confers its resistance and gemcitabine resistant cell line was found to have a 9 and 2-fold increase in RRM2 mRNA and protein expression respectively [27]. Whereas hydroxyurea targets RRM2, the overexpression of both RRM1 and RRM2 is associated with hydroxyurea resistance and the upregulation of RRM2 is more obvious [28]. These findings suggest that the two subunits play a role together in catalysis and regulation of drug resistance. However, unlike RRM1, whose expression nearly remains constant throughout the cell cycle, RRM2 usually increases during late G1 and early S phase of the cell cycle [29]. And during G2 phase, RRM2 will degrade via cyclin F which is following cyclin-dependent kinases (CDK)-mediated phosphorylation of Thr33 [30]. Therefore, RRM2 plays a dominant role in controlling RNR activity during the cell cycle. Besides, due to its more complex and various regulation ways than RRM1, RRM2 may be even more important than RRM1 in the occurrence of chemoresistance.

The studies found both mRNA and protein expression of RRM2 were high and ubiquitous in chemoresistance. The microarray of DOX- resistant human breast cancer cell found RRM2 gene was associated with DOX resistance [10]. Besides, the mRNA expression levels of RRM2 significantly increased in peripheral blood samples from imatinib-resistant patients [11]. Both mRNA and protein of RRM2 may act as a useful biomarker for selecting the good responders to chemotherapy for cancers. High RRM2 mRNA expression was correlated with low chemotherapy sensitivity and poor prognosis [31]. Similarly, in combination chemotherapy of docetaxel and gemcitabine, NSCLC patients with high levels of RRM2 mRNA had a significantly lower response rate, time to progression and overall survival [32,33]. Overexpression of RRM2 in tamoxifen-resistant patients was verified using tissue microarrays, which was also found in tamoxifen-resistant cells and xenograft tumors. And higher RRM2 protein expression was found in patients who did not respond to tamoxifen and associated significantly with earlier tumor recurrence [34]. Using multi-omics and bioinformatics analysis is easy to find the upregulation of RRM2 is associated with chemoresistance and poor prognosis, but the results still need to be verified in vivo and in vitro experiments and multi-center randomized controlled clinical trials [35].

3. Overexpression of RRM2 is mainly regulated by gene level, transcriptional level and post-transcriptional level

A protein could be upregulated by gene amplification, transcriptional activation, post-transcriptional regulation, and other unidentified mechanisms. As early as 1987, studies suggested that the gene of RRM2 might have been amplified in the selection of the hydroxyurea-resistant hamster and human cell lines [36] and rearranged in hydroxyurea-resistant mouse cells [37]. Furthermore, human RRM2 gene amplification from a homogeneous staining chromosome region always accompanies with altered transcription regulation. Gemcitabine-resistant and hydroxyurea-resistant clone exhibited a unique binding pattern with several transcription factors, including activator protein 1 (AP-1), Sp1, cAMP-response element binding protein (CREB) and nuclear factor kappa-B (NF-κB) [38]. Further research found that AP-1 could be induced by Vasohibin 2 (VASH2) and bind to the promoter of RRM2 to increase transactivation of RRM2, which was associated with gemcitabine resistance [39]. Checkpoint kinase 1 (Chk1) activation which induced by DNA damage can upregulate RRM2 expression through the E2F1 transcription factor [40]. Therefore, many chemicals like cyclin-dependent kinase inhibitor, Src tyrosine kinase inhibitor, and gambogic acid could synergy with gemcitabine by suppressing RRM2 expression via inhibiting E2F1 [41–43 Upregulation of nuclear factor Y (NF-Y) transactivation which is induced by the inhibition of histone deacetylases (HDAC) increases RRM2 transcription and contributes to gemcitabine resistance [44]. In addition, the activation of HIF-

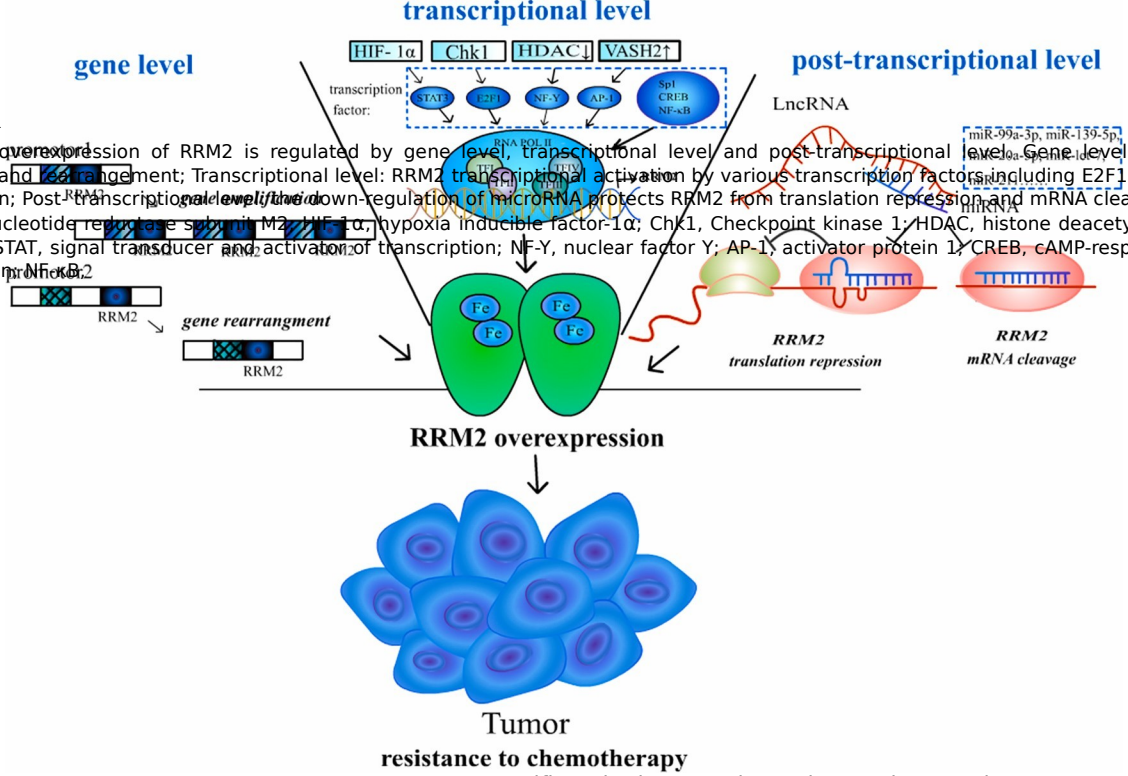

Fig. 1. The overexpression of RRM2 is regulated by gene level, transcriptional level and post-transcriptional level. Gene level: RRM2 gene amplification and rearrangement; Transcriptional level: RRM2 transcriptional activation by various transcription factors including E2F1, STAT3, NF-Y, AP-1 and so on; Post-transcriptional level: down-regulation of microRNA protects RRM2 from translation repression and mRNA cleavage. RRM2, Ribonucleotide reductase subunit M2; HIF-1α, hypoxia inducible factor-1α; Chk1, Checkpoint kinase 1; HDAC, histone deacetylases; VASH2, Vasohibin 2; STAT, signal transducer and activator of transcription; NF-Y, nuclear factor Y; AP-1, activator protein 1; CREB, cAMP-response element binding protein; NF-κB, nuclear factor kappa-B; LncRNA, Long non-coding RNA; miRNA, microRNA

1α/STAT3 signaling pathway can also upregulate RRM2 expression [45 The upregulation of RRM2 by transcriptional level in chemo-resistant cells is shown in Fig. 1.

Besides, more and more studies exhibited long non-coding RNA (lncRNA)/microRNA (miRNA) was also an important regulation way as miRNA could directly act on mRNA to mediate the post-transcriptional regulation. LncRNA AFAP1-AS1 promotes cell proliferation and chemoresistance in NSCLC through the upregulation of RRM2 by inhibiting miR-139− 5p [46]. LncRNA LINC00958 could regulate the expression of RRM2 by competing for miR-5095 [47]. It was found miR-99a-3p was significantly downregulated and its target gene, RRM2 was upregulated in sunitinib-resistant cells and clinical clear cell renal cell carcinoma tissues [48]. It was determined miR-20a-5p, miR-let-7, and miR-211 could inhibit the expression of RRM2 and increase the sensitivity to the gemcitabine in pancreatic cancer cells. In addition, miR-20a-5p and miR-let-7 could directly target the 3′untranslated region (3′UTR) of RRM2 mRNA [49–51]. Research also showed miR-4500 mediated RRM2 downregulation which could inhibit proliferation, angiogenesis, invasion, and migration of breast cancer cells and promoted apoptosis through inhibition of the MAPK signaling pathway [52]. Similarly, miR-140− 3p impeded the proliferation of human cervical cancer cells by targeting the 3′UTR of RRM2 mRNA [53]. It is now believed that miRNA "silences" target genes mainly through two ways. One is that miRNA is completely matched with the binding site in the 3′UTR region of target mRNA, which causes the cleavage of target mRNA, thus causing the rapid degradation of mRNA molecules. In the other case, incomplete base degradation is formed between miRNA and the 3′UTR region of mRNA, which leads to translation inhibition of target mRNA [54,55]. Other miRNA like miR-21 and miR-125b-5p also targets RRM2 in cancers, but their specific role in tumorigenesis or chemoresistance needs further research [56,57].

As above, the overexpression of RRM2 is regulated by gene level, transcriptional level, and post-transcriptional level, but there are still many regulating ways remain unknown and future research is needed urgently.

4. Overexpression of RRM2 facilitates DNA damage repair and influences cell signaling pathway to induce chemoresistance

As we know, uncontrolled proliferation of cancers needs a sufficient dNTP supply, so 200-fold differences in RNR activity were observed between fast- and slow-growing rat hepatoma cells [58]. RNR gene expression in human cancers has been surveyed using the ONCOMINE database. Remarkably, RRM2 was among the top 10 % most overexpressed genes in 73 out of the 168 cancer analyses [4]. Similarly, when DNA damage induced by chemotherapy occurs, the expression of RRM2 may be upregulated to increase the activity of RNR. Therefore, the overexpression of RRM2 coordinates with the S phase checkpoint in the absence of functional p53 to facilitate DNA damage repair and recovery from replication stress by supplying sufficient dNTPs [59]. It was suggested the combination treatment using everolimus and extracellular regulated protein kinases (ERK) inhibitors markedly attenuated the dNTPs pools by downregulating the mRNA expression of RRM1 and RRM2 through E2F1, thus synergistically inhibiting the proliferation of renal cell carcinoma cells [60]. Therefore, suppressing RRM2 expression or activity or both of them could interfere with the dNTPs pool, impeding DNA repair and replication to enhance the anticancer activity of chemotherapy especially DNA-damage drugs.

In another way, the increment of RRM2 would activate or inhibit signaling pathways that involve cell survival, proliferation, apoptosis, and so on. RRM2 suppression induces cell apoptosis through activation of the Bcl-2/caspase pathway and inhibits cell survival by suppressing the protein kinase B

(AKT) pathway in imatinib-resistant cells [11]. In addition, the upregulation of RRM2 also induces chemoresistance to cisplatin and fluorouracil (5-FU) via activating epidermal growth factor receptor (EGFR)/AKT proliferative pathway [46]. It was reported that elevated expression of RRM2 was associated with acquired tamoxifen resistance in breast cancer cells and inhibition of RRM2 significantly overturned this resistance [34,61]. Because overexpression of RRM2 leads to the upregulation of EGFR and NF-κB signaling pathway, downregulation of estrogen receptor (ER) α66, and upregulation of ERα36 (36-kDa variant of ER), contributing to the generation of acquired tamoxifen resistance [62]. And NF-κB is recognized as an important mediator of chemoresistance to agents including gemcitabine, as this pathway involves cell survival and proliferation [63].

Table 1
Inhibit the expression or activity of RRM2 to enhance the anticancer activity of chemotherapy.

The way to inhibit RRM2	Combinational anticancer drugs	cancers	Reference
SiRNA targets RRM2			
	cisplatin, 3-AP and hydroxyurea	human colon cancer	[64]
	gemcitabine	pancreatic and pancreatic ductal adenocarcinoma	[65]
	sunitinib	clear cell renal cell carcinoma	[48]
	imatinib	human myeloid leukemia	[11]
	gemcitabine and cisplatin	ovarian cancer	[66]
	doxorubicin	pancreatic cancer and hepatocellular carcinoma	[68]
	temozolomide	melanoma and glioblastoma	[69]
	mutant BRAF inhibitors, PLX4720	melanoma	[94]
RRM2 inhibitors			
Hydroxyurea	temozolomide	glioblastoma	[75]
COH29	hydroxyurea and gemcitabine	many cancers such as ovarian cancer and leukemia	[76]
Didox	sunitinib and tamoxifen	clear cell renal cell carcinoma and breast cancer.	[48]
Deferasirox	gemcitabine	pancreatic cancer	[79]
GW8510	gemcitabine and tamoxifen	lung squamous cell carcinoma and breast cancer cells	[80]
DHS	gemcitabine and cisplatin	pancreatic cancer and ovarian cancer	[82]
Kinases inhibitors	gemcitabine		[41]
Flavopiridol(cyclin-dependent kinase inhibitor)		pancreatic, gastric and colon cancer	
PP2 (Src tyrosine kinase inhibitor)	gemcitabine	pancreatic adenocarcinoma	[42]
ABC294640 (sphingosine kinase-2 inhibitor)	gemcitabine	pancreatic cancer	[83]
LY2603618 (Chk1 inhibitor)	gemcitabine	pancreatic cancer	[84]
Mocetinostat (class I HDAC inhibitor)	gemcitabine	leiomyosarcoma	[85]
MI-63 (murine double minute protein-2 inhibitor)	gemcitabine	mantle cell lymphoma	[86]
Others			
Bran ethanol extract	gemcitabine	pancreatic cancer	[89]
Gambogic acid	gemcitabine	pancreatic cancer	[43]
Astaxanthin	gemcitabine	pancreatic cancer	[45]
NDAT(a derivative of tetraiodothyroacetic acid)	resveratrol	colorectal cancer	[91]
Pegylated arginine deiminase	gemcitabine	pancreatic cancer	[92]

5. Enhance the anticancer activity of chemotherapy by suppressing RRM2 expression or activity

In order to search for more effective treatment of cancers, exploring the upstream and downstream regulatory pathway of RRM2 as well as the role and mechanism of RRM2 in chemoresistance is essential, which could provide the theoretical support for drug development. As shown in Table 1, in this part we mainly discuss the means including small interfering RNA (siRNA), RRM2 inhibitors, kinase inhibitors and others which was reported could enhance the anticancer activity of chemotherapy by suppressing RRM2 expression or activity.

5.1. Small interfering RNA

It was reported that the siRNA-mediated decrement of the RRM2 markedly increased the sensitivity of p53(-/-)human colon cancer cells to the DNA-damage agent cisplatin as well as to the RRM2 inhibitors 3- AP and hydroxyurea [64], and enhanced pancreatic adenocarcinoma chemosensitivity to gemcitabine [65]. After that, RRM2-targeted gene therapy gained more attention and was applied to various cancers combined with other antitumor drugs. Sunitinib is the most common primary molecular-targeted agent for metastatic clear cell renal cell carcinoma. SiRNA against RRM2 exhibited anticancer effects in the sunitinib-resistant cells [48]. Imatinib-based targeted treatment is the standard therapy for chronic myeloid leukemia. RRM2 suppression by siRNA enhanced the apoptosis and the sensitivity to imatinib of imatinib-resistant human myeloid leukemia cell line [11]. SiRNA targeting RRM2 combined with gemcitabine or cisplatin could effectively improve the apoptosis rate in cisplatin-resistant ovarian cancer cells [66]. Besides, the experimental study of nude mice also determined RRM2 gene silencing combined with cisplatin was more likely to inhibit the growth of human ovarian cancer [67]. Combinational application of siRNA against RRM2 and DOX dramatically enhanced the antitumor efficacy by approximate 4 times more than applying DOX alone in pancreatic cancer cells and significantly suppressed the tumor growth in vivo too [68]. Synergistic inhibition of melanoma cell proliferation was also achieved by combination therapy of RRM2 siRNA and temozolomide [69].

Compared with direct application of siRNA, liposomes and nanoparticles have been used as good carriers because they can increase drug solubility, prolong drug duration in vivo, and achieve targeted delivery to reduce side effects and improve drug

resistance [70]. So co-delivery of anticancer drugs and siRNA targeting RRM2 to tumors maybe a better way than direct applying gene therapy. For instance, a designed cationic liposomes containing gemcitabine and siRNA targeting RRM2 could be delivered to pancreatic carcinoma cells and effectively increase the chemosensitivity of gemcitabine [71]. Gao, J and his colleagues also developed a new liposome-delivery system, which delivered DOX and RRM2 siRNA to EGFR overexpressing hepatocellular carcinoma cells, resulting in enhanced cytotoxicity, apoptosis, and senescence-inducing activity compared with single-drug loaded or non-targeted controls [72].

5.2. RRM2 inhibitors

Different from siRNA against RRM2 which effectively inhibits the protein expression level of RRM2, RRM2 inhibitors suppress RRM2 activity by destroying the essential diiron tyrosyl radical center using radical scavengers or iron chelators to inactivate RNR [73]. As mentioned in the previous review [74], among the RRM2 inhibitors evaluated, GTI-2040, gallium nitrate, and 3,4-dihydroxy-benzohydroxamic acid (Didox) are more efficacious as a monotherapy, whereas 3-AP is more efficacious as a combination agent. Hydroxyurea is currently used more in combination therapy, even though it is efficacious as a monotherapy. This part, we focus on the RRM2 inhibitors that used in combination chemotherapy to deal with chemoresistance.

Through screening of known anticancer agents on temozolomide- resistant glioblastoma cells, it was identified that hydroxyurea as well as siRNA against RRM2 and other RRM2 inhibitors could sensitize newly diagnosed and recurrent glioblastoma to temozolomide [75]. A new RRM2 inhibitor, COH29, which binds to a novel pocket located at the RRM1 and RRM2 interface, can overcome hydroxyurea- and gemcitabine- resistance in cancer cells like ovarian cancer and leukemia, although it can't suppress the expression of RRM2 [76]. Didox, a derivative of hydroxyurea, exhibits anticancer effects in the sunitinib-resistant renal cell carcinoma [48]. Besides, it prevents the emergence of tamoxifen-resistant populations and reverses the acquired tamoxifen resistance by inhibition of RRM2 and NF-κB signaling pathway in breast cancer [62]. Additionally, Didox synergistically enhances the antitumor effect of temozolomide [77] and carmustine in malignant gliomas [78].

Some novel RRM2 inhibitors not only suppress RNR activity but also the RRM2 protein expression levels. Deferasirox, an oral iron chelator, which could significantly down-regulate the RRM1 and RRM2 protein expression levels shows antiproliferative activity in pancreatic cancer cells and potentiates the effect of gemcitabine by decreasing the competition between gemcitabine and dNTPs [79]. Similarly, another RRM2 inhibitor, GW8510, suppresses the survival of lung squamous cell carcinoma cells and reverses the resistance of gemcitabine by inducing DNA damage and cell apoptosis through RRM2 downregulation-mediated autophagy induction [80]. And GW8510 restores the sensitivity of breast cancer cells to tamoxifen in the same way [81]. It was identified a synthetic analog of resveratrol (3,5,4′-trihydroxy-trans-stilbene), termed DHS (trans− 4,4′-dihydroxystilbene), that induced cyclin F-mediated downregulation of RRM2 by the proteasome. Thus, DHS inactivated RNR, inhibited DNA replication, induced DNA damage, promoted cell apoptosis, and overcame both gemcitabine resistance in pancreatic cancer and cisplatin resistance in ovarian cancer [82].

5.3. Kinases inhibitors

Some kinases inhibitors can suppress RRM2 expression and synergy with chemotherapeutic drugs. E2F1, an important transcription factor that regulates RRM2 transcription, could be suppressed by many kinases inhibitors. For example, flavopiridol, a cyclin-dependent kinase inhibitor, enhances the apoptosis induced by gemcitabine in human pancreatic, gastric, and colon cancer cell lines, which is associated with suppressing expression of the RRM2 by downregulating E2F1 [41]. Src tyrosine kinase inhibitor, 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2) can enhance anticancer activity of gemcitabine in the same way [42]. ABC294640, an inhibitor of sphingosine kinase-2, increases the expression of p21 which binds to CDK/Cyclin-D1 complexes to prevent the phosphorylation of Rb, and hypo phosphorylated Rb binds to E2F1 and prevents its transcriptional activity [83]. LY2603618, a specific Chk1 inhibitor, may reduce RRM1 and RRM2 accumulation via activation of CDK [84]. In another way, some pathway inhibitors like the class I HDAC inhibitor, mocetinostat, which plus gemcitabine exhibited a synergistic effect in cancers via reducing the expression of RRM1 and RRM2, but the mechanism is unclear [85]. The murine double minute protein-2 (HDM-2) inhibitor, MI-63, suppresses the expression of the RRM2 and enhances the antiproliferative and proapoptotic effects of the gemcitabine in wtp53 mantle cell lymphoma cell lines [86]. Wee1-selective inhibitor AZD1775 synergistically promotes ATR-selective inhibitor VE-822 induced antileukemic activity by decreasing number of cells in the G2/M phase through RRM2 inhibition [87]. A proteomic study determined when LCL161, a Smac's small molecule mimetic, combined with caspase inhibitors could promote the apoptosis of drug-resistant breast cancer cells by downregulation of RRM2, CDK4 and ITGB1 expression [88]. The specific mechanism of inhibiting RRM2 expression by the above inhibitors remains to be further studied.

5.4. Others

Chinese herbal medicines can effectively treat cancer patients, improve patient outcomes, and reduce the side effects of chemotherapeutic drugs [89]. Some of them can also suppress RRM2 expression to enhance the anticancer activity of chemotherapy. A recent study demonstrated bran ethanol extract could inhibit the viability of gemcitabine-resistant cells by downregulation of RRM1 and RRM2 mRNA, and combination therapy also showed synergistic effects on inhibition of colony formation and promotion of apoptosis [90]. Gambogic acid is one of the main components of gamboge, which is secreted by the garcinia hanburyi tree. It could suppress the expression of RRM2 by inhibiting

the ERK / E2F1 pathway and sensitize pancreatic cancer cells to the gemcitabine in vitro and in vivo [43].

Astaxanthin is a lipophilic compound, exhibiting antioxidant, anti-inflammatory, and immunomodulatory characteristics. It was found able to restore the sensitivity of pancreatic cancer cells to gemcitabine via downregulating RRM1 and RRM2 through regulating HIF- 1α/STAT3 pathway [45. NDAT is a nanoparticulate derivative of tetraiodothyroacetic acid, which exerts anticancer properties and downregulates RRM2 gene expression in cancer cells. RRM2 downregulation, whether achieved by RNA interference or treatment with NDAT, enhances resveratrol-induced antiproliferation [91]. Pegylated arginine deiminase which decreases extracellular arginine has been shown as a targeted therapeutic agent in the treatment of several types of cancer. And it synergistically increased the anticancer activity of gemcitabine [92]. Pancreatic adenocarcinoma up-regulated factor (PAUF) was increased in pancreatic cancer stem cells and the suppression of PAUF enhanced chemotherapeutic response to gemcitabine and 5-FU by inhibiting RRM2 [93].

6. Conclusion

RNR is the only enzyme that catalyzes de novo formation of deoxyribonucleotides, and the regulation of RRM2 expression has stronger temporality and spatiality than RRM1. Besides, the overexpression of RRM2 is common in tumorigenesis and chemoresistance. So we have reasons to believe RRM2 plays a crucial role in chemoresistance and serves as a potential target of chemotherapy whether used alone or combined with other drugs. Most chemotherapeutic drugs act by incorporation, interaction, or methylation of DNA to inhibit DNA replication or induce DNA damage so they are called DNA-damage agents. It was reported that inhibition of RNR by 3-AP enhanced radiation-mediated antitumor activity independent of p53 regulation by impairing repair processes that relies on dNTPs supply, thereby substantially increasing the radiation sensitivity in human cancers [9596]. Similarly, inactivating RNR by inhibition of RRM2 prevents dNTPs supply for DNA damage repair, promoting the apoptosis process initiated by anticancer agents. Another important class of chemotherapeutic drugs, molecular targeted drugs like tyrosine kinase inhibitors and receptor antagonists have a higher potency in terms of anti-proliferation and anti-angiogenesis. Inhibiting the expression of RRM2 probably could also interact with the EGFR/AKT proliferative pathway, the Bcl-2/caspase apoptotic pathway, and other signaling pathways, thus exerts synergistic anticancer activity with molecular targeted drugs [11, 46]. Besides, when you inhibit the activity of RRM2 which decreasing dNTPs synthesis, the competition between gemcitabine or other deoxycytidine analogs, and deoxycytidine would also be decreased, thus the anticancer activity would be enhanced [79,86].

Different from some RRM2 inhibitors like 3-AP which has carried out many clinical trials [13–17], many novel ways to inhibit RRM2 expression or activity only focus on basic researches at the cellular and animal levels but lack clinical experience. SiRNA or kinase inhibitors which inhibit the expression of RRM2 may be a potential adjuvant therapy in the treatment of cancer, when combined with other chemotherapeutic drugs, as the dose of drugs required to kill cancer cells decreased and the side effects of drugs can simultaneously decreased in the clinic. But there is still a long way to go for clinical use. SiRNA can efficiently trigger specific genes silencing, and more and more emerging technologies have been used to improve pharmacokinetics, pharmacodynamics or limit toxicity, but a large number of challenges involving safety and potency still cannot be ignored [97]. The effectiveness and safety of various kinase inhibitors and Chinese herbal medicine also need to be verified in clinical trials. As for RRM2 inhibitors, there is still a lot of work to be done before new RRM2 inhibitors were found with high effectivity and exactly used in the clinic, whether it is a single drug use or adjuvant chemotherapy. However, exploring the mechanism of RRM2 in chemotherapy resistance is still helpful to fully understand the pathophysiological function of RRM2 and to find new therapeutic targets.

In conclusion, inhibition of RRM2 expression or activity may be a vital way to enhance the efficacy of chemotherapy via inhibiting DNA damage repair and regulating signaling pathways about cell survival, apoptosis, proliferation, and so on. SiRNA, RRM2 inhibitors, kinases inhibitors, and other chemicals indeed effectively suppress the expression level or activity of RRM2 in cell lines and animal models, and promise to be a new anticancer treatment either as a single agent or combined with other therapies.

Ethical

appro

val

Not

require

d

Fundi

ng

This review was funded by National Natural Science Foundation of China (No. 81971982) and Zhejiang Provincial Science and Technology Department (No.2020C03046)

Declaration of Competing Interest

The authors report no declarations of interest.

References

[1] U. Uhlin, H. Eklund, Structure of ribonucleotide reductase protein R1, Nature 370 (6490) (1994) 533–539.
[2] H. Tanaka, H. Arakawa, T. Yamaguchi, K. Shiraishi, S. Fukuda, K. Matsui, Y. Takei, Y. Nakamura, A ribonucleotide reductase gene involved in a p53-dependent cell- cycle checkpoint for DNA damage, Nature 404 (6773) (2000) 42–49.
[3] G. Pontarin, P. Ferraro, L. Bee, P. Reichard, V. Bianchi, Mammalian ribonucleotide reductase subunit p53R2 is

required for mitochondrial DNA replication and DNA repair in quiescent cells, Proc. Natl. Acad. Sci. U. S. A. 109 (33) (2012) 13302–13307.
[4] Y. Aye, M. Li, M.J. Long, R.S. Weiss, Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies, Oncogene 34 (16) (2015) 2011–2021.
[5] J. Shao, X. Liu, L. Zhu, Y. Yen, Targeting ribonucleotide reductase for cancer therapy, Expert Opin. Ther. Targets 17 (12) (2013) 1423–1437.
[6] S. Nyholm, L. Thelander, A. Graslund, Reduction and loss of the iron center in the¨ reaction of the small subunit of mouse ribonucleotide reductase with hydroxyurea, Biochemistry 32 (43) (1993) 11569–11574.
[7] L. Zhu, B. Zhou, X. Chen, H. Jiang, J. Shao, Y. Yen, Inhibitory mechanisms of heterocyclic carboxaldehyde thiosemicabazones for two forms of human ribonucleotide reductase, Biochem. Pharmacol. 78 (9) (2009) 1178–1185.
[8] A.A. Desai, R.L. Schilsky, A. Young, L. Janisch, W.M. Stadler, N.J. Vogelzang, S. Cadden, J.A. Wright, M.J. Ratain, A phase I study of antisense oligonucleotide GTI-2040 given by continuous intravenous infusion in patients with advanced solid tumors, Anal. oncol.: off. J. European Soc. Med. Oncol. 16 (6) (2005) 958–965.
[9] R. Danesi, G. Altavilla, E. Giovannetti, R. Rosell, Pharmacogenomics of gemcitabine in non-small-cell lung cancer and other solid tumors, Pharmacogenomics 10 (1) (2009) 69–80.
[10] S. Zhou, J. Li, H. Xu, S. Zhang, X. Chen, W. Chen, S. Yang, S. Zhong, J. Zhao, J. Tang, Liposomal curcumin alters chemosensitivity of breast cancer cells to Adriamycin via regulating microRNA expression, Gene 622 (2017) 1–12.
[11] C. Liu, Y. Li, R. Hu, W. Han, S. Gao, Knockdown of ribonucleotide reductase regulatory subunit M2 increases the drug sensitivity of chronic myeloid leukemia to imatinibbased therapy, Oncol. Rep. 42 (2) (2019) 571–580.
[12] Y. Yen, K. Margolin, J. Doroshow, M. Fishman, B. Johnson, C. Clairmont, D. Sullivan, M. Sznol, A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer, Cancer Chemother. Pharmacol. 54 (4) (2004) 331–342.
[13] A. Mortazavi, Y. Ling, L.K. Martin, L. Wei, M.A. Phelps, Z. Liu, E.J. Harper, S.P. Ivy, X. Wu, B.S. Zhou, X. Liu, D. Deam, J.P. Monk, W.J. Hicks, Y. Yen, G.A. Otterson, M. R. Grever, T. Bekaii-Saab, A phase I study of prolonged infusion of triapine in combination with fixed dose rate gemcitabine in patients with advanced solid tumors, Invest. New Drugs 31 (3) (2013) 685–695.
[14] C. Kunos, T. Radivoyevitch, F.W. Abdul-Karim, J. Fanning, O. Abulafia, A. J. Bonebrake, L. Usha, Ribonucleotide reductase inhibition restores platinum-sensitivity in platinum-resistant ovarian cancer: a gynecologic oncology group study, J. Transl. Med. 10 (2012) 79.
[15] L.K. Martin, J. Grecula, G. Jia, L. Wei, X. Yang, G.A. Otterson, X. Wu, E. Harper, C. Kefauver, B.S. Zhou, Y. Yen, M. Bloomston, M. Knopp, S.P. Ivy, M. Grever, T. Bekaii-Saab, A dose escalation and pharmacodynamic study of triapine and radiation in patients with locally advanced pancreas cancer, Int. J. Radiat. Oncol. Biol. Phys. 84 (4) (2012) e475–81.
[16] C.A. Kunos, S. Waggoner, V. von Gruenigen, E. Eldermire, J. Pink, A. Dowlati, T. J. Kinsella, Phase I trial of pelvic radiation, weekly cisplatin, and 3-aminopyridine- 2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) for locally advanced cervical cancer, Clin. cancer res.: off. J. American Assoc. Cancer Res. 16 (4) (2010) 1298–1306.
[17] C.A. Kunos, T. Radivoyevitch, S. Waggoner, R. Debernardo, K. Zanotti, K. Resnick, N. Fusco, R. Adams, R. Redline, P. Faulhaber, A. Dowlati, Radiochemotherapy plus 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) in advanced-stage cervical and vaginal cancers, Gynecol. Oncol. 130 (1) (2013) 75–80.
[18] W.M. Stadler, A.A. Desai, D.I. Quinn, R. Bukowski, B. Poiesz, C.G. Kardinal, N. Lewis, A. Makalinao, P. Murray, F.M. Torti, A Phase I/II study of GTI-2040 and capecitabine in patients with renal cell carcinoma, Cancer Chemother. Pharmacol. 61 (4) (2008) 689–694.
[19] N.B. Leighl, S.A. Laurie, X.E. Chen, P. Ellis, F.A. Shepherd, J.J. Knox, G. Goss, R. L. Burkes, G.R. Pond, C. Dick, Y. Yen, J.A. Zwiebel, M.J. Moore, A phase I/II study of GTI-2040 plus docetaxel as second-line treatment in advanced non-small cell lung cancer: a study of the PMH phase II consortium, J. thoracic oncol.: off. Pub. Int. Assoc. Study Lung Cancer 4 (9) (2009) 1163–1169.
[20] S.S. Sridhar, C.M. Canil, K.N. Chi, S.J. Hotte, S. Ernst, L. Wang, E.X. Chen, A. Juhasz, Y. Yen, P. Murray, J.A. Zwiebel, M.J. Moore, A phase II study of the antisense oligonucleotide GTI-2040 plus docetaxel and prednisone as first-line treatment in castration-resistant prostate cancer, Cancer Chemother. Pharmacol. 67 (4) (2011) 927–933.
[21] L. Malik, A. Zwiebel, J. Cooper, A phase I pharmacokinetic and pharmacodynamic study of GTI-2040 in combination with gemcitabine in patients with solid tumors, Cancer Chemother. Pharmacol. 82 (3) (2018) 533–539.
[22] R.B. Klisovic, W. Blum, Z. Liu, Z. Xie, C. Kefauver, L. Huynh, J.A. Zwiebel, S. M. Devine, J.C. Byrd, M.R. Grever, K.K. Chan, G. Marcucci, Phase I study of GTI- 2040, a ribonucleotide reductase antisense, with high dose cytarabine in patients with relapsed/refractory acute myeloid leukemia, Leuk. Lymphoma 55 (6) (2014) 1332–1336.
[23] Y. Binenbaum, S. Na'ara, Z. Gil, Gemcitabine resistance in pancreatic ductal adenocarcinoma, Drug Resist. Updat. 23 (2015) 55–68.
[24] J. Carmichael, The role of gemcitabine in the treatment of other tumours, Br. J. Cancer 78 (Suppl 3) (1998) 21–25.
[25] A.M. Bergman, H.M. Pinedo, G.J. Peters, Determinants of resistance to 2',2'- difluorodeoxycytidine (gemcitabine), Drug resist. updates: rev. commentaries antimicrob. anticancer chemother. 5 (1) (2002) 19–33.
[26] C. Wang, W. Zhang, M. Fu, A. Yang, H. Huang, J. Xie, Establishment of human pancreatic cancer gemcitabineresistant cell line with ribonucleotide reductase overexpression, Oncol. Rep. 33 (1) (2015) 383–390.
[27] Y.G. Goan, B. Zhou, E. Hu, S. Mi, Y. Yen, Overexpression of ribonucleotide reductase as a mechanism of resistance to 2,2-difluorodeoxycytidine in the human KB cancer cell line, Cancer Res. 59 (17) (1999) 4204–4207.
[28] G.A. McClarty, A.K. Chan, Y. Engstrom, J.A. Wright, L. Thelander, Elevated expression of M1 and M2 components and drug-induced posttranscriptional modulation of ribonucleotide reductase in a hydroxyurea-resistant mouse cell line, Biochemistry 26 (24) (1987) 8004–8011.
[29 pression of mammalian ribonucleotide reductase. Differential regulation of the two subunits, J. Biol. Chem. 260 (16) (1985) 9114–9116.
[30] V. D'Angiolella, V. Donato, F.M. Forrester, Y.T. Jeong, C. Pellacani, Y. Kudo, A. Saraf, L. Florens, M.P. Washburn, M. Pagano, Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair, Cell 149 (5) (2012) 1023–1034.
[31] T. Itoi, A. Sofuni, N. Fukushima, F. Itokawa, T. Tsuchiya, T. Kurihara, F. Moriyasu, A. Tsuchida, K. Kasuya, Ribonucleotide reductase subunit M2 mRNA expression in pretreatment biopsies obtained from unresectable pancreatic carcinomas, J. Gastroenterol. 42 (5) (2007) 389–394.
[32] J. Souglakos, I. Boukovinas, M. Taron, P. Mendez, D. Mavroudis, M. Tripaki, D. Hatzidaki, A. Koutsopoulos, E. Stathopoulos, V. Georgoulias, R. Rosell, Ribonucleotide reductase subunits M1 and M2 mRNA expression levels and clinical outcome of lung adenocarcinoma patients treated with docetaxel/gemcitabine, Br. J. Cancer 98 (10) (2008) 1710–1715.
[33] I. Boukovinas, C. Papadaki, P. Mendez, M. Taron, D. Mavroudis, A. Koutsopoulos, M. Sanchez-Ronco, J.J. Sanchez, M. Trypaki, E. Staphopoulos, V. Georgoulias, R. Rosell, J. Souglakos, Tumor BRCA1, RRM1 and RRM2 mRNA expression levels and clinical response to first-line gemcitabine plus docetaxel in non-small-cell lung cancer patients, PLoS One 3 (11) (2008) e3695.
[34] N. Putluri, S. Maity, R. Kommagani, C.J. Creighton, V. Putluri, F. Chen, S. Nanda, S. K. Bhowmik, A. Terunuma, T. Dorsey, A. Nardone, X. Fu, C. Shaw, T.R. Sarkar, R. Schiff, J.P. Lydon, B.W. O'Malley, S. Ambs, G.M. Das, G. Michailidis, A. Sreekumar, Pathway-centric integrative analysis identifies RRM2 as a prognostic marker in breast cancer associated with poor survival and tamoxifen resistance, Neoplasia (New York, N.Y.) 16 (5) (2014) 390–402.
[35] R. Liu, C.X. Guo, H.H. Zhou, Network-based approach to identify prognostic biomarkers for estrogen receptor-positive breast cancer treatment with tamoxifen, Cancer Biol. Ther. 16 (2) (2015) 317–324.

[36] P.N. Tonin, R.L. Stallings, M.D. Carman, J.R. Bertino, J.A. Wright, P.R. Srinivasan, W.H. Lewis, Chromosomal assignment of amplified genes in hydroxyurea-resistant hamster cells, Cytogenet. Cell Genet. 45 (2) (1987) 102–108.
[37] Y. Wettergren, A. Kullberg, G. Levan, Drug-specific rearrangements of chromosome 12 in hydroxyurea-resistant mouse SEWA cells: support for chromosomal breakage model of gene amplification, Somat. Cell Mol. Genet. 20 (4) (1994) 267–285.
[38] B. Zhou, X. Mo, X. Liu, W. Qiu, Y. Yen, Human ribonucleotide reductase M2 subunit gene amplification and transcriptional regulation in a homogeneous staining chromosome region responsible for the mechanism of drug resistance, Cytogenet. Cell Genet. 95 (1–2) (2001) 34–42.
[39] M. Tu, H. Li, N. Lv, C. Xi, Z. Lu, J. Wei, J. Chen, F. Guo, K. Jiang, G. Song, W. Gao, Y. Miao, Vasohibin 2 reduces chemosensitivity to gemcitabine in pancreatic cancer cells via Jun proto-oncogene dependent transactivation of ribonucleotide reductase regulatory subunit M2, Mol. Cancer 16 (1) (2017) 66.
[40] Y.W. Zhang, T.L. Jones, S.E. Martin, N.J. Caplen, Y. Pommier, Implication of checkpoint kinase-dependent up-regulation of ribonucleotide reductase R2 in DNA damage response, J. Biol. Chem. 284 (27) (2009) 18085–18095.
[41] C.P. Jung, M.V. Motwani, G.K. Schwartz, Flavopiridol increases sensitization to gemcitabine in human gastrointestinal cancer cell lines and correlates with down-regulation of ribonucleotide reductase M2 subunit, Clin. cancer res.: off. J. American Assoc. Cancer Res. 7 (8) (2001) 2527–2536.
[42] M.S. Duxbury, H. Ito, M.J. Zinner, S.W. Ashley, E.E. Whang, Inhibition of SRC tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells, Clin. cancer res.: off. J. American Assoc. Cancer Res. 10 (7) (2004) 2307–2318.
[43] G. Xia, H. Wang, Z. Song, Q. Meng, X. Huang, X. Huang, Gambogic acid sensitizes gemcitabine efficacy in pancreatic cancer by reducing the expression of ribonucleotide reductase subunit-M2 (RRM2), J. Exp. Clin. Cancer Res. 36 (1) (2017) 107.
[44] X. Liu, B. Zhou, L. Xue, W. Qiu, J. Shih, S. Zheng, Y. Yen, Nuclear factor Y regulation and promoter transactivation of human ribonucleotide reductase subunit M2 gene in a Gemcitabine resistant KB clone, Biochem. Pharmacol. 67 (8) (2004) 1499–1511.
[45] T. Yan, H.Y. Li, J.S. Wu, Q. Niu, W.H. Duan, Q.Z. Han, W.M. Ji, T. Zhang, W. Lv, Astaxanthin inhibits gemcitabine-resistant human pancreatic cancer progression through EMT inhibition and gemcitabine resensitization, Oncol. Lett. 14 (5) (2017) 5400–5408.
[46 N. Huang, W. Guo, K. Ren, W. Li, Y. Jiang, J. Sun, W. Dai, W. Zhao, LncRNA AFAP1- AS1 supresses miR-139-5p and promotes cell proliferation and chemotherapy resistance of non-small cell lung Cancer by competitively upregulating RRM2, Front. Oncol. 9 (2019) 1103.
[47] H. Zhao, G.H. Zheng, G.C. Li, L. Xin, Y.S. Wang, Y. Chen, X.M. Zheng, Long noncoding RNA LINC00958 regulates cell sensitivity to radiotherapy through RRM2 by binding to microRNA-5095 in cervical cancer, J. Cell. Physiol. 234 (12) (2019) 23349–23359.
[48] Y. Osako, H. Yoshino, T. Sakaguchi, S. Sugita, M. Yonemori, M. Nakagawa, H. Enokida, Potential tumorsuppressive role of microRNA99a3p in sunitinibresistant renal cell carcinoma cells through the regulation of RRM2, Int. J. Oncol. 54 (5) (2019) 1759–1770.
[49] H. Lu, S. Lu, D. Yang, L. Zhang, J. Ye, M. Li, W. Hu, MiR-20a-5p regulates gemcitabine chemosensitivity by targeting RRM2 in pancreatic cancer cells and serves as a predictor for gemcitabine-based chemotherapy, Biosci. Rep. 39 (5) (2019).
[50] Y.D. Bhutia, S.W. Hung, M. Krentz, D. Patel, D. Lovin, R. Manoharan, J. M. Thomson, R. Govindarajan, Differential processing of let-7a precursors influences RRM2 expression and chemosensitivity in pancreatic cancer: role of LIN- 28 and SET oncoprotein, PLoS One 8 (1) (2013), e53436.
[51] M. Maftouh, A. Avan, N. Funel, A.E. Frampton, H. Fiuji, S. Pelliccioni, L. Castellano, V. Galla, G.J. Peters, E. Giovannetti, miR-211 modulates gemcitabine activity through downregulation of ribonucleotide reductase and inhibits the invasive behavior of pancreatic cancer cells, Nucleosides, Nucleotides & nucleic acids 33 (4–6) (2014) 384–393.
[52] S. Li, H. Mai, Y. Zhu, G. Li, J. Sun, G. Li, B. Liang, S. Chen, MicroRNA-4500 inhibits migration, invasion, and angiogenesis of breast Cancer cells via RRM2-Dependent MAPK signaling pathway, Mol. Ther. Nucleic Acids 21 (2020) 278–289.
[53] J. Ma, F. Zhang, P. Sun, miR-140-3p impedes the proliferation of human cervical cancer cells by targeting RRM2 to induce cell-cycle arrest and early apoptosis, Bioorg. Med. Chem. 28 (3) (2020), 115283.
[54] C.J. Stavast, S.J. Erkeland, The non-canonical aspects of MicroRNAs: many roads to gene regulation, Cells 8 (11) (2019).
[55] B. Liu, J. Li, M.J. Cairns, Identifying miRNAs, targets and functions, Brief. Bioinformatics 15 (1) (2014) 1–19.
[56] D. Quan, K. Chen, J. Zhang, Y. Guan, D. Yang, H. Wu, S. Wu, L. Lv, Identification of lncRNA NEAT1/miR-21/RRM2 axis as a novel biomarker in breast cancer, J. Cell. Physiol. 235 (4) (2020) 3372–3381.
[57] S.P. Huang, Y.F. Jiang, L.J. Yang, J. Yang, M.T. Liang, H.F. Zhou, J. Luo, D.P. Yang, W.J. Mo, G. Chen, L. Shi, T.Q. Gan, Downregulation of miR-125b-5p and its prospective molecular mechanism in lung squamous cell carcinoma, Cancer Biother. Radiopharm. (2020).
[58] H.L. Elford, M. Freese, E. Passamani, H.P. Morris, Ribonucleotide reductase and cell proliferation. I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas, J. Biol. Chem. 245 (20) (1970) 5228–5233.
[59] Z.P. Lin, M.F. Belcourt, R. Carbone, J.S. Eaton, P.G. Penketh, G.S. Shadel, J. G. Cory, A.C. Sartorelli, Excess ribonucleotide reductase R2 subunits coordinate the S phase checkpoint to facilitate DNA damage repair and recovery from replication stress, Biochem. Pharmacol. 73 (6) (2007) 760–772.
[60 Y. Zou, W. Li, J. Zhou, J. Zhang, Y. Huang, Z. Wang, ERK inhibitor enhances everolimus efficacy through the attenuation of dNTP pools in renal cell carcinoma, Mol. Ther. Nucleic Acids 14 (2019) 550–561.
[61 K.N. Shah, K.R. Mehta, D. Peterson, M. Evangelista, J.C. Livesey, J.S. Faridi, AKT- induced tamoxifen resistance is overturned by RRM2 inhibition, Mol. Cancer Res. 12 (3) (2014) 394–407.
[62] K.N. Shah, E.A. Wilson, R. Malla, H.L. Elford, J.S. Faridi, Targeting ribonucleotide reductase M2 and NF-kappaB activation with Didox to circumvent tamoxifen resistance in breast Cancer, Mol. Cancer Ther. 14 (11) (2015) 2411–2421.
[63] A. Arlt, A. Gehrz, S. Müerkoster, J. Vorndamm, M.L. Kruse, U.R. ¨ Folsch, H. Sc¨ hafer,¨ Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death, Oncogene 22 (21) (2003) 3243–3251.
[64 Z.P. Lin, M.F. Belcourt, J.G. Cory, A.C. Sartorelli, Stable suppression of the R2 subunit of ribonucleotide reductase by R2-targeted short interference RNA sensitizes p53(-/-) HCT-116 colon cancer cells to DNA-damaging agents and ribonucleotide reductase inhibitors, J. Biol. Chem. 279 (26) (2004) 27030–27038.
[65] M.S. Duxbury, H. Ito, M.J. Zinner, S.W. Ashley, E.E. Whang, RNA interference targeting the M2 subunit of ribonucleotide reductase enhances pancreatic adenocarcinoma chemosensitivity to gemcitabine, Oncogene 23 (8) (2004) 1539–1548.
[66] M. Zhang, J. Wang, R. Yao, L. Wang, Small interfering RNA (siRNA)-mediated silencing of the M2 subunit of ribonucleotide reductase: a novel therapeutic strategy in ovarian cancer, Int. J. gynecological cancer: off. J. Int. Gynecological Cancer Soc. 23 (4) (2013) 659–666.
[67] T. Xue, L. Wang, Y. Li, H. Song, H. Chu, H. Yang, A. Guo, J. Jiao, SiRNA-mediated RRM2 gene silencing combined with cisplatin in the treatment of epithelial ovarian Cancer In vivo: an experimental study of nude mice, Int. J. Med. Sci. 16 (11) (2019) 1510–1516.
[68] S. Zheng, X. Wang, Y.H. Weng, X. Jin, J.L. Ji, L. Guo, B. Hu, N. Liu, Q. Cheng, J. Zhang, H. Bai, T. Yang, X.H. Xia, H.Y. Zhang, S. Gao, Y. Huang, siRNA knockdown of RRM2 effectively suppressed pancreatic tumor growth alone or synergistically with doxorubicin, Mol. Ther. Nucleic Acids 12 (2018) 805–816.
[69] J.E. Zuckerman, T. Hsueh, R.C. Koya, M.E. Davis, A. Ribas, siRNA knockdown of ribonucleotide reductase inhibits melanoma cell line proliferation alone or synergistically with temozolomide, J. Invest. Dermatol. 131 (2) (2011) 453–460.
[70] Y. Liu, W.L. Lu, Q. Zhang, [Recent advances in liposomes and nanoparticles as drug carriers for drug delivery], Zhongguo yi xue ke xue yuan xue bao, Acta Academiae Medicinae Sinicae 28 (4) (2006) 583–589.

[71] X. Zhao, X. Wang, W. Sun, K. Cheng, H. Qin, X. Han, Y. Lin, Y. Wang, J. Lang, R. Zhao, X. Zheng, Y. Zhao, J. Shi, J. Hao, Q.R. Miao, G. Nie, H. Ren, Precision design of nanomedicines to restore gemcitabine chemosensitivity for personalized pancreatic ductal adenocarcinoma treatment, Biomaterials 158 (2018) 44–55.
[72] J. Gao, H. Chen, Y. Yu, J. Song, H. Song, X. Su, W. Li, X. Tong, W. Qian, H. Wang, J. Dai, Y. Guo, Inhibition of hepatocellular carcinoma growth using immunoliposomes for co-delivery of adriamycin and ribonucleotide reductase M2 siRNA, Biomaterials 34 (38) (2013) 10084–10098.
[73] J. Shao, B. Zhou, B. Chu, Y. Yen, Ribonucleotide reductase inhibitors and future drug design, Curr. Cancer Drug Targets 6 (5) (2006) 409–431.
[74] M.B. Mannargudi, S. Deb, Clinical pharmacology and clinical trials of ribonucleotide reductase inhibitors: is it a viable cancer therapy? J. Cancer Res. Clin. Oncol. 143 (8) (2017) 1499–1529.
[75] J. Teng, S. Hejazi, L. Hiddingh, L. Carvalho, M.C. de Gooijer, H. Wakimoto, M. Barazas, M. Tannous, A.S. Chi, D.P. Noske, P. Wesseling, T. Wurdinger, T. T. Batchelor, B.A. Tannous, Recycling drug screen repurposes hydroxyurea as a sensitizer of glioblastomas to temozolomide targeting de novo DNA synthesis, irrespective of molecular subtype, Neuro-oncology 20 (5) (2018) 642–654.
[76] B. Zhou, L. Su, S. Hu, W. Hu, M.L. Yip, J. Wu, S. Gaur, D.L. Smith, Y.C. Yuan, T. W. Synold, D. Horne, Y. Yen, A small-molecule blocking ribonucleotide reductase holoenzyme formation inhibits cancer cell growth and overcomes drug resistance, Cancer Res. 73 (21) (2013) 6484–6493.
[77] M. Figul, A. Soling, H.J. Dong, T.C. Chou, N.G. Rainov, Combined effects of¨ temozolomide and the ribonucleotide reductase inhibitors didox and trimidox in malignant brain tumor cells, Cancer Chemother. Pharmacol. 52 (1) (2003) 41–46. [78] Z. Horvath, W. Bauer, T. Hoechtl, P. Saiko, M. Fritzer-Szekeres, T. Tihan, T. Szekeres, Combination chemotherapy of BCNU and Didox acts synergystically in 9L glioma cells, Nucleosides, nucleotides & nucleic acids 23 (8-9) (2004) 1531–1535.
[79] S. Shinoda, S. Kaino, S. Amano, H. Harima, T. Matsumoto, K. Fujisawa, T. Takami, N. Yamamoto, T. Yamasaki, I. Sakaida, Deferasirox, an oral iron chelator, with gemcitabine synergistically inhibits pancreatic cancer cell growth in vitro and in vivo, Oncotarget 9 (47) (2018) 28434–28444.
[80] P. Chen, J.N. Wu, Y. Shu, H.G. Jiang, X.H. Zhao, H. Qian, K. Chen, T. Lan, C. G. Chen, J. Li, Gemcitabine resistance mediated by ribonucleotide reductase M2 in lung squamous cell carcinoma is reversed by GW8510 through autophagy induction, Clin. sci. (London, England: 1979) 132 (13) (2018) 1417–1433.
[81] Z.N. Li, Y. Shu, C.G. Chen, X.Q. Li, M.Y. Li, X.H. Zhao, S. Wang, J. Li, Acquired tamoxifen resistance is surmounted by GW8510 through ribonucleotide reductase M2 downregulation-mediated autophagy induction, Biochem. Biophys. Res. Commun. (2020).
[82] C.W. Chen, Y. Li, S. Hu, W. Zhou, Y. Meng, Z. Li, Y. Zhang, J. Sun, Z. Bo, M. L. DePamphilis, Y. Yen, Z. Han, W. Zhu, (trans-4,4′-dihydroxystilbene) suppresses DNA replication and tumor growth by inhibiting RRM2 (ribonucleotide reductase regulatory subunit M2), Oncogene 38 (13) (2019) 2364–2379.
[83] C.S. Lewis, C. Voelkel-Johnson, C.D. Smith, Suppression of c-Myc and RRM2 expression in pancreatic cancer cells by the sphingosine kinase-2 inhibitor ABC294640, Oncotarget 7 (37) (2016) 60181–60192.
[84] M. Liang, T. Zhao, L. Ma, Y. Guo, CHK1 inhibition sensitizes pancreatic cancer cells to gemcitabine via promoting CDK-dependent DNA damage and ribonucleotide reductase downregulation, Oncol. Rep. 39 (3) (2018) 1322–1330.
[85] G. Lopez, D. Braggio, A. Zewdu, L. Casadei, K. Batte, H.K. Bid, D. Koller, P. Yu, O. H. Iwenofu, A. Strohecker, E. Choy, D. Lev, R. Pollock, Mocetinostat combined with gemcitabine for the treatment of leiomyosarcoma: preclinical correlates, PLoS One 12 (11) (2017), e0188859.
[86] R.J. Jones, V. Baladandayuthapani, S. Neelapu, L.E. Fayad, J.E. Romaguera, M. Wang, R. Sharma, D. Yang, R.Z. Orlowski, HDM-2 inhibition suppresses expression of ribonucleotide reductase subunit M2, and synergistically enhances gemcitabine-induced cytotoxicity in mantle cell lymphoma, Blood 118 (15) (2011) 4140–4149.
[87] W. Qi, X. Xu, M. Wang, X. Li, C. Wang, L. Sun, D. Zhao, L. Sun, Inhibition of Wee1 sensitizes AML cells to ATR inhibitor VE-822-induced DNA damage and apoptosis, Biochem. Pharmacol. 164 (2019) 273–282.
[88] G. Jin, K. Wang, Y. Liu, X. Liu, X. Zhang, H. Zhang, Proteomic level changes on treatment in MCF-7/DDP breast Cancer Drug-Resistant cells, Anticancer Agents Med. Chem. (2020).
[89] Z. Wang, X. Liu, R.L. Ho, C.W. Lam, M.S. Chow, Precision or personalized medicine for Cancer chemotherapy: is there a role for herbal medicine, Molecules 21 (7) (2016).
[90] M. Kim, J.G. Mun, H.J. Lee, S.R. Son, M.J. Lee, J.Y. Kee, Inhibitory effect of oat bran ethanol extract on survival and gemcitabine resistance of pancreatic Cancer cells, Molecules 24 (21) (2019).
[91] A.W. Nana, S.Y. Wu, Y.S. Yang, Y.T. Chin, T.M. Cheng, Y. Ho, W.S. Li, Y.M. Liao, Y. R. Chen, Y.J. Shih, Y.R. Liu, J. Pedersen, S. Incerpi, A. Hercbergs, L.F. Liu, J. Whang-Peng, P.J. Davis, H.Y. Lin, Nano-diamino-Tetrac (NDAT) enhances resveratrol-induced antiproliferation by action on the RRM2 pathway in colorectal cancers, Horm. Cancer 9 (5) (2018) 349–360.
[92] R. Daylami, D.J. Muilenburg, S. Virudachalam, R.J. Bold, Pegylated arginine deiminase synergistically increases the cytotoxicity of gemcitabine in human pancreatic cancer, J. Exp. Clin. Cancer Res. 33 (1) (2014) 102.
[93] J.H. Cho, S.A. Kim, S.B. Park, H.M. Kim, S.Y. Song, Suppression of pancreatic adenocarcinoma upregulated factor (PAUF) increases the sensitivity of pancreatic cancer to gemcitabine and 5FU, and inhibits the formation of pancreatic cancer stem like cells, Oncotarget 8 (44) (2017) 76398–76407.
[94] N. Fatkhutdinov, K. Sproesser, C. Krepler, Q. Liu, P.A. Brafford, M. Herlyn, K. M. Aird, R. Zhang, Targeting RRM2 and mutant BRAF is a novel combinatorial strategy for melanoma, Mol. cancer res.: MCR 14 (9) (2016) 767–775.
[95] C.A. Kunos, S.M. Chiu, J. Pink, T.J. Kinsella, Modulating radiation resistance by inhibiting ribonucleotide reductase in cancers with virally or mutationally silenced p53 protein, Radiat. Res. 172 (6) (2009) 666–676.
[96] C.A. Kunos, T. Radivoyevitch, J. Pink, S.M. Chiu, T. Stefan, J. Jacobberger, T. J. Kinsella, Ribonucleotide reductase inhibition enhances chemoradiosensitivity of human cervical cancers, Radiat. Res. 174 (5) (2010) 574–581.
[97] R.L. Setten, J.J. Rossi, S.P. Han, The current state and future directions of RNAi- based therapeutics, Nature reviews, Drug discovery 18 (6) (2019) 421–446.